

Figure 1

# Activation of the Transcription Factor NF- $\kappa$ B through TNF Receptor 2 in CT6 Cells

	Prelimmune		Anti-mTNF-R2		Prelimmune		Anti-mTNF-R2		Prelimmune		Anti-mTNF-R2	
NF- $\kappa$ B Probe	wt	wt	mt	mt	wt	wt	wt	wt	wt	wt	wt	wt
Competitor	-	-	-	-	mt	mt	mt	mt	AP-1	AP-1	AP-1	AP-1

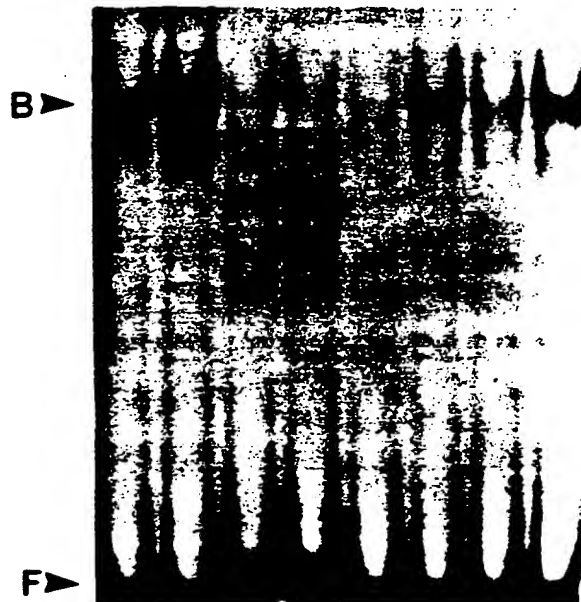


Figure 2

## Immunoprecipitation of Human TNF Receptor 2

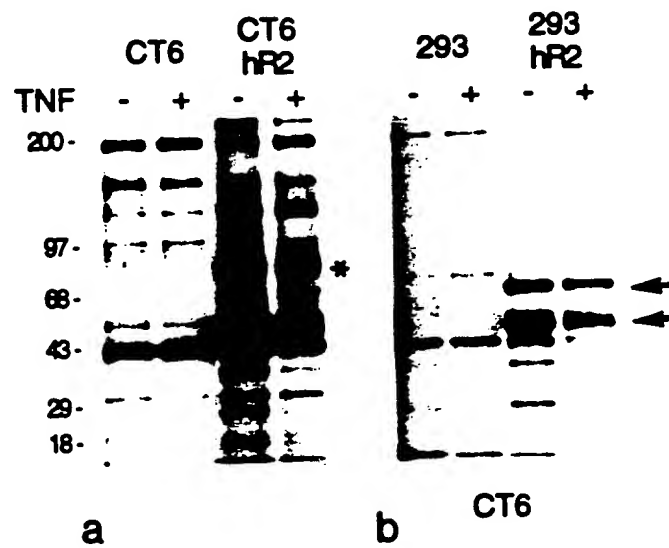


Figure 3

Glutathione-S-Transferase human TNF Receptor 2  
Intracellular Domain Fusion Protein

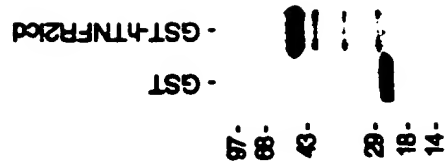
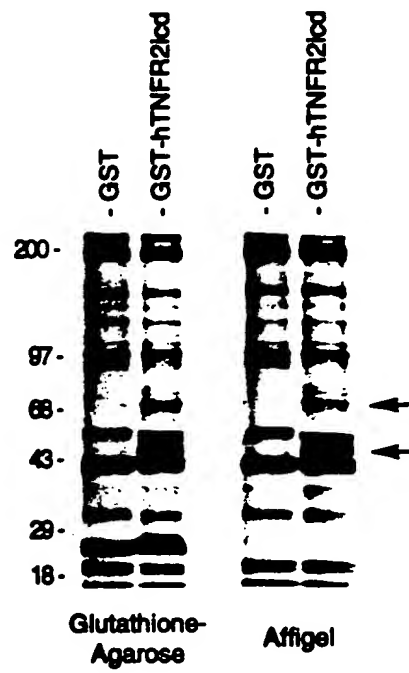


Figure 4

Coprecipitation of Glutathione-S-Transferase  
Human TNF Receptor 2 Intracellular Domain  
Fusion Protein in CT6 Cell Extracts



Coprecipitation of Glutathione-S-Transferase Mutant  
Human TNF Receptor 2 Intracellular Domain  
Fusion Proteins in CT6 Cell Extracts

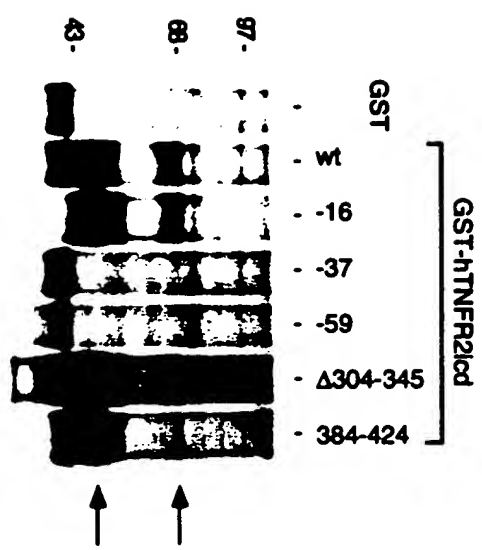


Figure 5

# Competition of TNF Receptor 2 Associated Factors with Glutathione-S-Transferase TNF Receptor 2 Intracellular Domain Fusion Proteins

Figure 6

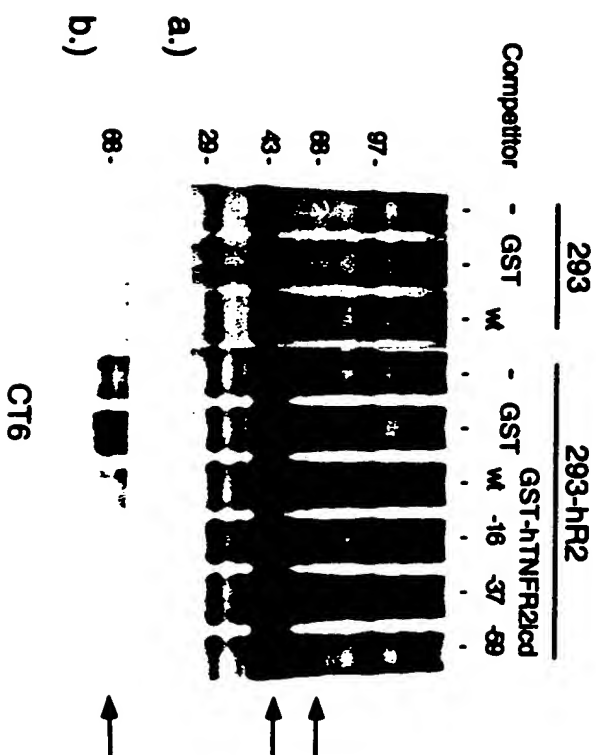


Figure 7

**Coprecipitation of Glutathione-S-Transferase  
Human TNF Receptor 2 Intracellular Domain  
Fusion Protein in Jurkat Cell Extracts**

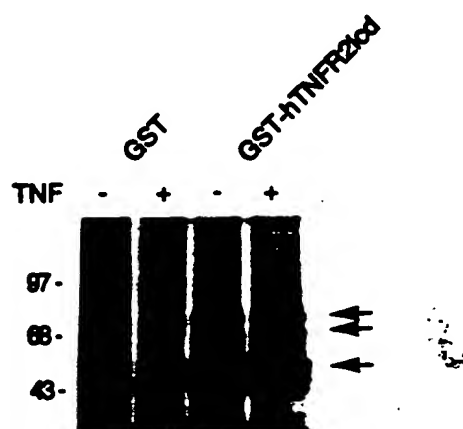


Figure 8

### Intracellular Localization of TNF Receptor 2 Associated Factors

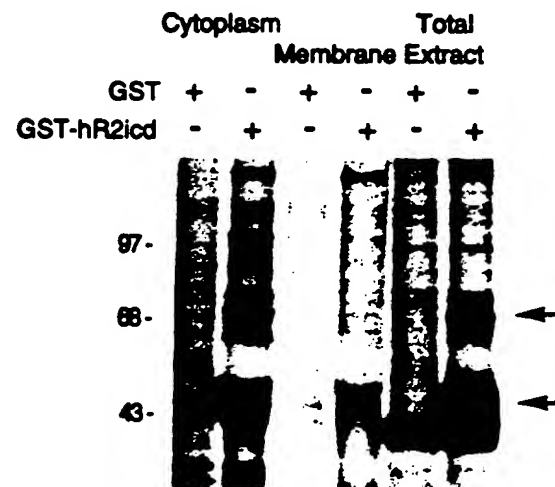
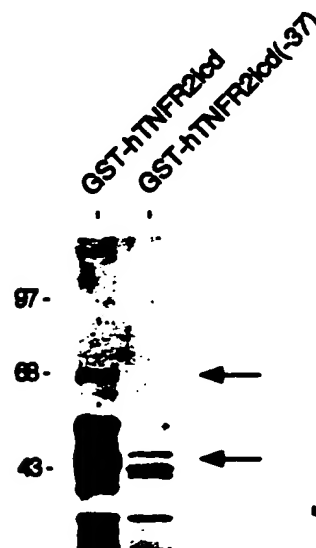




Figure 9

Purification of TNF Receptor 2  
Associated Factors



1 CCCAGCCCGGTTCTCTGCCCAAGGACGCTACCGCCCAATGCGAGCAGAAGGCGGCGCACAGATACAGAAAGT  
 74 GAGGCTCAGACATATTGAGACCGTGTGACATAGGGTAGCCAAATGACAGTGTGAGAAAGTGACATTACTCAAG  
 149 GCCACCCAGATATCTGGAGGACCCAGAACCTGGAGATTCCCATCAGAAAGACCTTCTGGCCACCTGAAACCCC  
 1 MetAlaSerSerSerAlaProAspGluAsnGluPheGlnPheGlyCysProProAlaProCysGlnAspPro  
 224 AAGATGGCCTCCAGCTCAGCCCCGTGATGAAAACGAGTTTCAATTTGGTTGCCCCCTGCTCCCTGCCAGGACCCA  
 25 SerGluProArgValLeuCysCysThrAlaCysLeuSerGluAsnLeuArgAspAspGluAspArgIleCysPro  
 299 TCGGAGCCCAGAGTTCTCTGCTGCACAGCCTGTCTCTCTGAGAACCTGAGAGATGATGAGGATCGGATCTGTCT  
 50 LysCysArgAlaAspAsnLeuHisProValSerProGlySerProLeuThrGlnGluLysValHisSerAspVal  
 374 AAATGCAGAGCAGACAACCTCCATCTGTGAGCCCAGGAAGCCCTCTGACTCAGGAGAAGGTTCACTCTGATGTA  
 75 AlaGluAlaGluIleMetCysProPheAlaGlyValGlyCysSerPheLysGlySerProGlnSerMetGlnGlu  
 449 GCTGAGGCTGAAATCATGTGCCCCCTTGCAGGTGTTGGCTGTTCTTCAAGGGAGCCCAATCCATGCAGGAG  
 100 HisGluAlaThrSerGlnSerSerHisLeuTyrLeuLeuAlaValLeuLysGluTrpLysSerSerProGly  
 524 CATGAGGCTACCTCCAGTCTCCACCTGTACCTGCTGCTGGCGGTCTTAAAGGAGTGGAATCCTCACCAGGC  
 125 SerAsnLeuGlySerAlaProMetAlaLeuGluArgAsnLeuSerGluLeuGlnLeuGlnAlaAlaValGluAla  
 599 TCCAACCTAGGGTCTGCACCCATGGCACTGGAGCGGAACCTGTGAGAGCTGCAGCTTCAGGCAGCTGTGGAAGCG  
 150 ThrGlyAspLeuGluValAspCysTyrArgAlaProCysCysGluSerGlnGluGluLeuAlaLeuGlnHisLeu  
 674 ACAGGGGACCTGGAGGTAGACTGCTACCGGGCACCTTGCTGTGAGAGCCAGGAAGAACTGGCCCTGCAGCACTTG  
 175 ValLysGluLysLeuLeuAlaGlnLeuGluGluLysLeuArgValPheAlaAsnIleValAlaValLeuAsnLys  
 749 GTGAAGGAGAAGCTGTGGCTCAGCTGGAGGAGAAGCTGCGTGTGTTGCAACATTGTTGCTGTCTCAACAG  
 200 GluValGluAlaSerHisLeuAlaLeuAlaAlaSerIleHisGlnSerGlnLeuAspArgGluHisLeuLeuSer  
 824 GAAGTGGAGGCTTCCACCTGGCACTGGCCGCTCCATCCACCAGAGCCAGTTGGACCGAGAGCACCTCCTGAGC  
 225 LeuGluGlnArgValValGluLeuGlnGlnThrLeuAlaGlnLysAspGlnValLeuGlyLysLeuGluHisSer  
 899 TTGGAGCAGAGGGTGGTGAATTACAGCAAACCTGGCTCAAAAAGACCAGGTCTGGGCAAGCTTGAGCACAGT  
 250 LeuArgLeuMetGluGluAlaSerPheAspGlyThrPheLeuTrpLysIleThrAsnValThrLysArgCysHis  
 974 CTGCGACTCATGGAGGAGGCATCCTTTGATGGTACTTCTCTGTTGAAGATCACCATGTACCAAGCGGTGCCAC  
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 300 LeuArgLeuTyrLeuAsnGlyAspGlySerGlyLysLysThrHisLeuSerLeuPheIleValIleMetArgGly  
 1124 CTGCGCTTGTACCTGAACGGGGATGGCTCAGGCAAGAAGACCCACCTGTCCCTCTTCATCGTGATCATGAGAGGA  
 325 GluTyrAspAlaLeuLeuProTrpProPheArgAsnLysValThrPheMetLeuLeuAspGlnAsnAsnArgGlu  
 1199 GAATACGATGCTCTCTGCCCTGGCCTTTAGGAACAAGGTACCTTTATGCTACTTGACCAGAACAACCGAGAG  
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 400 PheLeuLysCysIleValAspThrSerAla  
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 1724 AATGTTGAGACCAGCCTAGATCAGGATGAAAAGAGCCAGGCTGAGGCTTGACATTGAGCCAAGGCTATGGGGC  
 1799 CTAAGTGGAGGGGCACTCCTACCAGGACATTCTCTCGAGGTCAGGGCATAACTGGAAAAATGCCCCCATCTCTCT  
 1874 GTTCAGACTCAAACTAGAACCACAGGGCAGAAGGGTCAGACATTAATGTGAATTTAACTTGGCCCTGGACTGAGT  
 1949 TCCATGTGTTAACAGACACGCAACAGGTAAACCCAGAACTGCCCTGGGAAATGCTTTCTGGCTGCATCTGGAGA  
 2024 TCTTTGATGTTTTTACCGACAAAACAAATAACAAAGCCTTGAATTGCAAAAAAAAAAAAAAAAAA

Figure 10

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      * * *
1      MetAlaAlaAlaSerValThrSerPro
1      GCGCGAAGACCGTTGGGGCTTTGTGGTGTGTGGGGTTGTAACTCACATGGCTGCAGCCAGTGTGACTTCCCT
10 GlySerLeuGluLeuLeuGlnProGlyPheSerLysThrLeuLeuGlyThrArgLeuGluAlaLysTyrLeuCys
75 GGCTCCCTAGAACTGCTACAGCCTGGCTTCTCCAAGACCTCCTGGGGACCAGGTTAGAAGCCAAGTACCTCTGT
35 SerAlaCysLysAsnIleLeuArgArgProPheGlnAlaGlnCysGlyHisArgTyrCysSerPheCysLeuThr
150 TCAGCCTGCAAAAACATCCTGCGGAGGCCTTTCCAGGCCAGTGTGGGCACCGCTACTGCTCCTTCTGCTGACC
60 SerIleLeuSerSerGlyProGlnAsnCysAlaAlaCysValTyrGluGlyLeuTyrGluGluGlyIleSerIle
225 AGCATCCTCAGCTCTGGGCCCCAGAACTGTGCTGCCTGTGTCTATGAAGGCTGTATGAAGAAGGCATTTCTATT
85 LeuGluSerSerSerAlaPheProAspAsnAlaAlaArgArgGluValGluSerLeuProAlaValCysProAsn
300 TTAGAGAGTAGTTCCGGCCTTTCCAGATAACGCTGCCCGCAGAGAGGTGGAGAGCCTGCCAGCTGTCTGTCCCAAT
110 AspGlyCysThrTrpLysGlyThrLeuLysGluTyrGluSerCysHisGluGlyLeuCysProPheLeuLeuThr
375 GATGGATGCACTTGAAGGGGACCTTGAAAGAATACGAGAGCTGCCACGAAGGACTTTGCCCATTCCTGCTGACG
135 GluCysProAlaCysLysGlyLeuValArgLeuSerGluLysGluHisHisThrGluGlnGluCysProLysArg
450 GAGTGTCTGCATGTAAAGGCTGGTCCGCCTCAGCGAGAAGGAGCACCACACTGAGCAGGAATGCCCCAAAAGG
160 SerLeuSerCysGlnHisCysArgAlaProCysSerHisValAspLeuGluValHisTyrGluValCysProLys
525 AGCCTGAGCTGCCAGCACTGCAGAGCACCCTGTAGCCACGTGGACCTGGAGGTACACTATGAGGTCTGCCCAAG
185 PheProLeuThrCysAspGlyCysGlyLysLysLysIleProArgGluThrPheGlnAspHisValArgAlaCys
600 TTTCCCTTAACCTGTGTATGGCTGTGGCAAGAAGAAGATCCCTCGGGAGACGTTTCAGGACCATGTTAGAGCATGC
210 SerLysCysArgValLeuCysArgPheHisThrValGlyCysSerGluMetValGluThrGluAsnLeuGlnAsp
675 AGCAAATGCCGGGTTCTCTGCAGATTCCACACCGTTGGCTGTTTCAGAGATGGTGGAGACTGAGAACCTGCAGGAT
235 HisGluLeuGlnArgLeuArgGluHisLeuAlaLeuLeuLeuSerSerPheLeuGluAlaGlnAlaSerProGly
750 CATGAGCTGCAGCGGTACGGGAACACCTAGCCCTACTGCTGAGCTCATTCTTGGAGGCCCAAGCCTCTCCAGGA
260 ThrLeuAsnGlnValGlyProGluLeuLeuGlnArgCysGlnIleLeuGluGlnLysIleAlaThrPheGluAsn
825 ACCTTGAACAGGTGGGGCCAGAGCTACTCCAGCGGTGCCAGATTTTGGAGCAGAAGATAGCAACCTTTGAGAAC
285 IleValCysValLeuAsnArgGluValGluArgValAlaValThrAlaGluAlaCysSerArgGlnHisArgLeu
900 ATTGTCTGCGTCTGAACCGTGAAGTAGAGAGGTTAGCAGTGACTGCAGAGGCTTGTAGCCGGCAGCACC GGCTA
310 AspGlnAspLysIleGluAlaLeuSerAsnLysValGlnGlnLeuGluArgSerIleGlyLeuLysAspLeuAla
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385 ArgTyrGlyTyrLysMetCysLeuArgValTyrLeuAsnGlyAspGlyThrGlyArgGlyThrHisLeuSerLeu
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410 PhePheValValMetLysGlyProAsnAspAlaLeuLeuGlnTrpProPheAsnGlnLysValThrLeuMetLeu
1275 TTCTTCGTGGTGATGAAAGGCCCAATGATGCTCTGTTGAGTGGCCTTTTAAATCAGAAGGTAACATTGATGTTG
435 LeuAspHisAsnAsnArgGluHisValIleAspAlaPheArgProAspValThrSerSerSerPheGlnArgPro
1350 CTGGACCATAACAACCGGGAGCATGTGATCGACGCATTGAGGCCGATGTAACCTCGTCCTCCTCCAGAGGCCT
460 ValSerAspMetAsnIleAlaSerGlyCysProLeuPheCysProValSerLysMetGluAlaLysAsnSerTyr
1425 GTCAGTGACATGAACATCGCCAGTGGCTGCCCCCTCTTCTGCCCTGTGTCCAAGATGGAGGCCAAGAATTCCTAT
485 ValArgAspAspAlaIlePheIleLysAlaIleValAspLeuThrGlyLeu
1500 GTGCGGGATGATGCGATCTTCATCAAAGCTATTGTGGACCTAACAGGACTCTAGCCACCCCTGCTAAGAATAGCA
1575 GCTCAGTGAGGAGCTGTACATTAGGCCAGCCAGGCCCTGCCACACACGGGTGGGCAGGCTTGGTGTAATAGCTG
1650 GGGAGGGCCTCAGCCTAGAGCCAATCACCATCACACAGAAAGGCAGGAAGAAGCCTCCAGTTGGCCTTCAGCTGG
1725 CAAACTGAGTTGGACGCTCACTGAGCTCAAGGGCCTGGTGGAGCCCGCTGGGGAGCTTCTCAGCTTTCCAATAG
1800 GAAAGCTCCTGCTGTCTCTCTGTCTGGGAAGGGAGAGACCTGTAGGTGGGTGCTCAGAAAGGGCCTCTCCAGA
1875 GAGAGTCTCAAGAGCTGCAGCAGGAGCAAGTGACTGGCCTTCCCCACCCATCCTTTGGAAAAGAGGTAGCGGC
1950 TACACAGGAGAAGGCATGCGCCTGCAGGGTGTAGCCCAAGAGAGAAGCTCTCTGAGACATAGGCCCTCACTGGAG
2025 AAGGGCCTGCCTGGGCTGCACAGCCTTGCCAGGTGGCCTGTATGGGGAGAAGTGATTAAATGTTGAGATGTCAC
2100 ACGACAAAAA

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Figure 11

A

Figure 12a

TRAF2	(mouse)	31	KYLCSACKNILRRPFQA QCGHRYCSFCLTISI	LSS	GPQNCACVYE
COP1	(A. thaliana)	49	DLLCPICMQI IKDAFLT ACGHSFCymCIITH	LRN	KSDCPCCSQH
EPF	(human)	10	ELSCSICLEPFKEPVTTP CGHNFCSCLNETWA	VQG	SPYLCPQCRAY
RAD-18	(S. cerevisiae)	25	LLRCHICKDFLKVPLVT PCGHTFCSLCIRTH	LNN	QPNCPCLFE
UVS-2	(N. crassa)	31	AFRCHVCKDFYDSPMLT SCNHTPCSLCIRRC	LSV	DSK CPLCRAT
RAG-1	(human)	290	SISCQICEHILADPVEET NCKHVFCRVCILRC	LKV	MGSYCPSCRYP
SS-A/Ro	(human)	13	EVTCPICLDPFVEPVSIECGHSFCQECISQV	GKG	GGSVCAVCRQR
RING1	(human)	16	ELMCPICLDMLKNTMTTKECLEHRCSDCIYTA	LRS	GNKECPTCRKK
RPT-1	(mouse)	12	EVTCPICLELKEPVSA DCNHSFCRACITLNTYESNRNTDGKGNCPVCRVP		
RFP	(human)	13	ETTCPVCLQYFAEPMML DCGHNICCAICLARCWGT	ETT	VS CPQCRET
c-cbl	(human)	378	FQLCKICAENDKVKIE PCGHLMCTSCLT	WQSE	SEQ GSSGCPFCRCE
consensus			---C-C-----X11-12	X10-16	---C-C---

# B

TRAF2	(mouse)	157	CPKRSLS <sup>C</sup> QHC RAPCSHV <sup>D</sup> LEV <sup>H</sup> YE VC
		182	PKFPL <sup>T</sup> CDG <sup>C</sup> GKKKIPRET <sup>F</sup> QD <sup>H</sup> VR AC
DG17	(D. discoideum)	171	GGFKLV <sup>T</sup> CD <sup>F</sup> C <sup>C</sup> KRDDIKKKLE <sup>T</sup> H <sup>H</sup> YK TC
TFIIIA	(X. laevis)	189	QD LAV <sup>C</sup> DV <sup>C</sup> NRRKFRHKDYLRD <sup>H</sup> QK TH
XLCOF14	(X. laevis)	1	TGKYPT <sup>C</sup> SEC <sup>C</sup> GKSFMDKRYLKI <sup>H</sup> SN VH
XFIN	(X. laevis)	1225	TGEKPY <sup>T</sup> CT <sup>V</sup> C <sup>C</sup> GKKFIDRSSVVK <sup>H</sup> SR TH
ZFY1/2	(mouse)	521	RKKFPH <sup>T</sup> CGE <sup>C</sup> GKGFRRHPSALKK <sup>H</sup> IR VH
MFG2	(mouse)	293	SEKPFEC <sup>E</sup> EE <sup>C</sup> GKKFRFARHLVK <sup>H</sup> QR IH
RAD18	(S. cerevisiae)	183	PNEQMAQ <sup>C</sup> PI <sup>C</sup> QGFYPLKALEKT <sup>H</sup> LD EC
UVS-2	(N. crassa)	182	PDDGLVA <sup>C</sup> PI <sup>C</sup> L <sup>T</sup> RM KEQGVDR <sup>H</sup> LD <sup>T</sup> SC

Figure 12b

TRAF2 1 MAAASVTSPGSELELLQPGFSKTLTGTRLEAKYLCBCKNILRRPFAQCG

TRAF2 51 HRYCSFCLTSILSSGPQNCACVYEGLYEEGISILESSAFPDAARREV

TRAF2 101 ESLPAVCPNDGCTWKGTLEKEYESCHEGLCPFLLTECPACKGLVRLSEKEN  
TRAF1 1 ..... MASSAPDENEFOFGCPPA

TRAF2 151 HTEQECPRKRLSCQHCRAPCSHVDLEVHYEVCCKFPLTCDGCQKKKIPRE  
TRAF1 20 PCODPSEPRLVLCCTACLSENLRDDIEDRICPKCRADMLHPVSPD-SPLTQE

TRAF2 201 TFQDMVIRACSKCRVLCRFHTVGCSEMVETENLQDHQLRLREHLALLSS  
TRAF1 69 KVMSDV...AEAEIMCPFAGVGCSEFKGSPQSMOEHEATSOSSHLVLLAV

TRAF2 251 FLEAOASPGTLNOVGPELLO.....  
TRAF1 116 LKEWKS SPGSNLGSAPMALERNLSELOQA AVEATQDLEVDCYRAPCCES

TRAF2 272 .....COILEQKIATFENIVCVLNREVERVAVTAECSSRH  
TRAF1 166 QEELALQHLVKEKLLAQLEEKLRVFANIVAVLNKEVEASHLALAASIMHS

TRAF2 308 RLDGDKIEALSNNKVOQLERSIGLKDAMADLEOKVSELVSTYDGVFIWK  
TRAF1 216 OLDREHLLSLEQRYVELOOTLAGKDOVLGKLEHSLRLMEASFDGTFLWK

TRAF2 358 ISDFTRKRQEA VAGRTPAIFSPAFTYSRYGYKMCLRVYLNQDGTGRGTHL  
TRAF1 266 ITNVTKRCHESVCGRTVSLFSPAFTYAKYGYKLCRLYLNQDGS GKKT HL

TRAF2 408 SLFFVVMKGPNDALLQWPFNQKVTLMLLDHNNREHVDAFRPDVTSSSFO  
TRAF1 316 SLFIVIMRGEYDALLPWPFRNKVTFMLLDONNREHAIDAFRPLSSASFO

TRAF2 458 RPVS DMNIASGCPLFCPVSKME-AKNSYVRDDAIFIKAIVDLTGL  
TRAF1 366 RPOSETNV ASGCPLFFPLSKLOSPKHAYVKDDTMFLKCI VDTSA

Figure 13

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08/779596

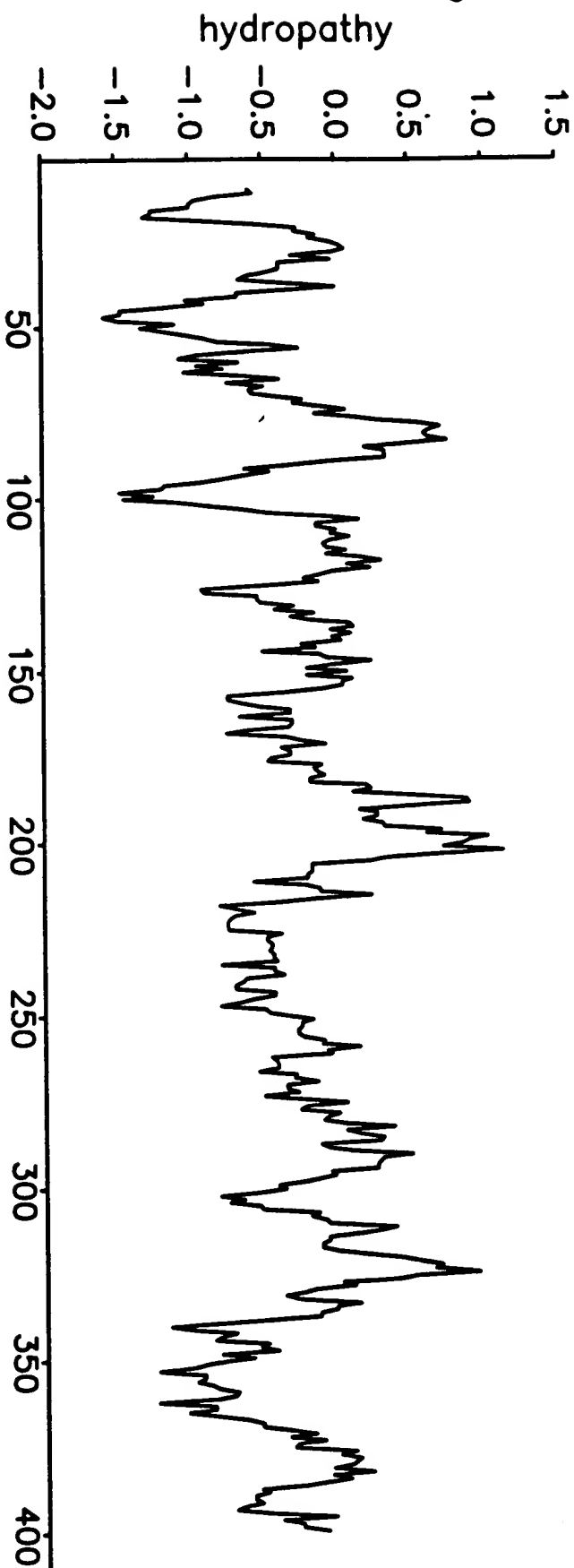


Figure 14a

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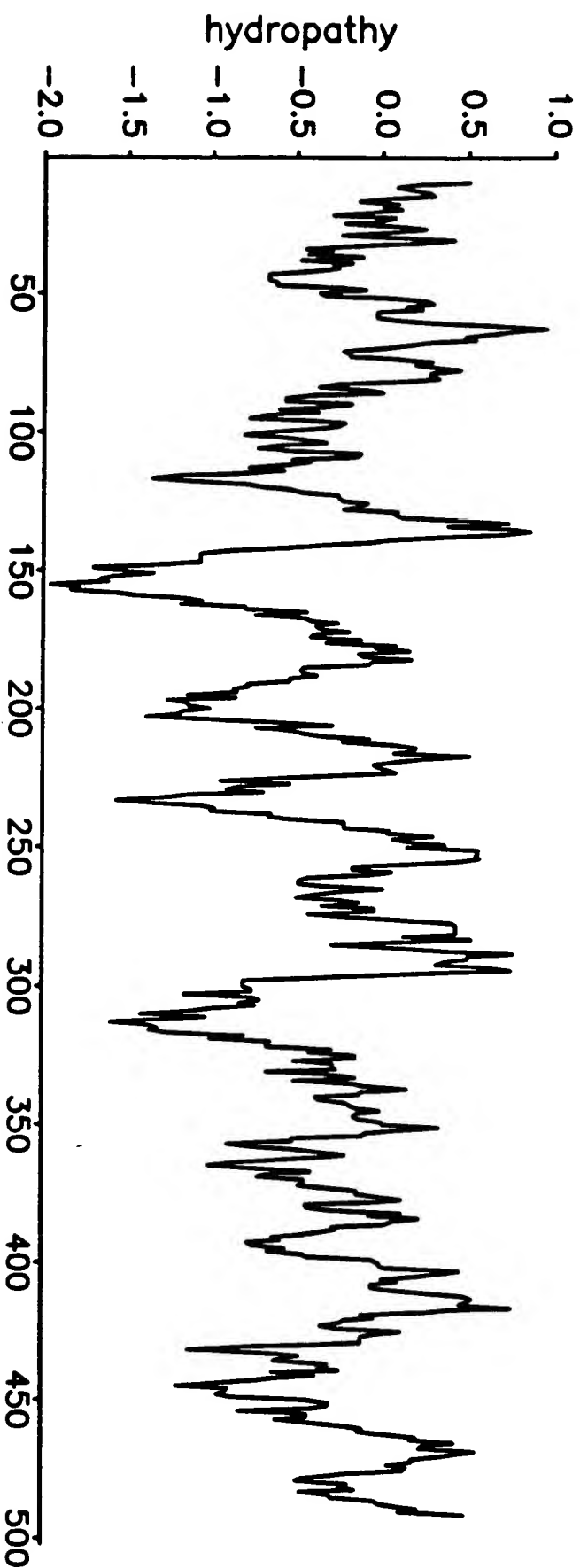


Figure 14b



# TRAF Expression in CT6 Cells

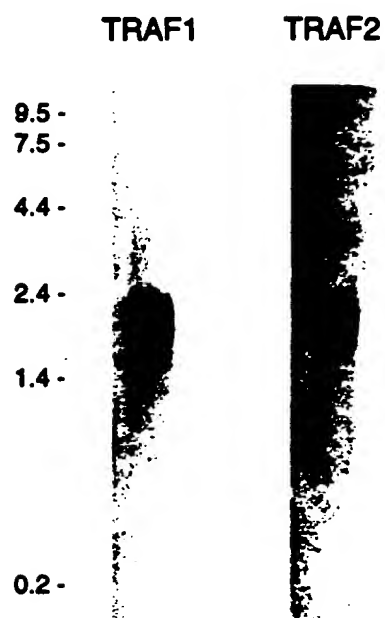


Figure 15a

Figure 15b

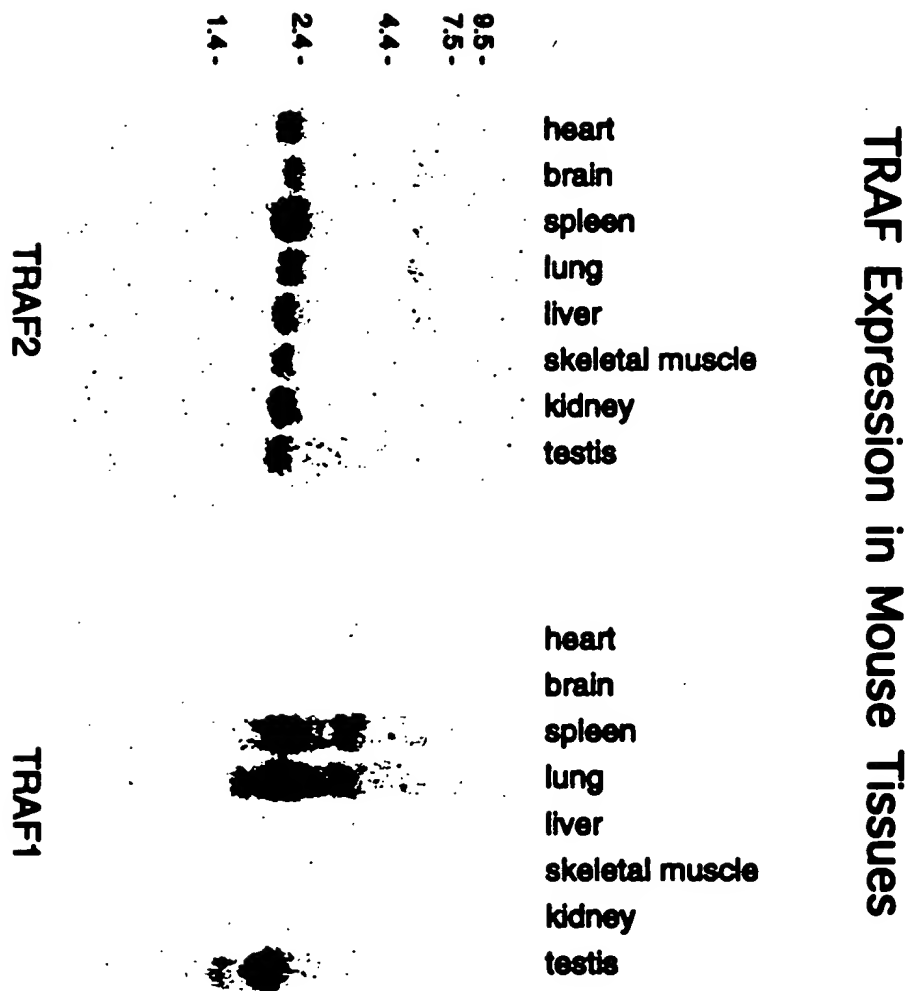


Figure 16

**A Glutathione-S-Transferase TRAF2 Fusion Protein  
Coprecipitates the Human TNF-R2 in 293 Cell Extracts**

